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(54) METHOD OF POTENTIATING THE ACTIVITY OF PHOSPHONOMYCIN ANTIBIOTICS

(71) We, MERCK & CO. INC., a corporation duly organised and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed to be particularly described in and by the following statement:—

The elimination of bacterial infections by antibiotic therapy is often thwarted by practical difficulties for achieving sufficiently high levels of the antibiotic at the site of infection. In cases where only marginally effective concentrations are used, antibiotic-resistant organisms frequently emerge from the original infecting population. This problem is important in the case of the new antibiotic phosphonomycin [(−) (*cis* - 1,2 - epoxypropyl) phosphonic acid] since it is excreted rapidly and its action is antagonized by common constituents of plasma and urine such as glucose and phosphate, respectively. In addition, the antibiotic is occasionally found to be ineffective against pre-existent mutants which are relatively resistant to this antibiotic and occur within many bacterial populations. Accordingly, methods of overcoming these difficulties in antibiotic therapy have been sought.

This present invention is based on the discovery that the activity of phosphonomycin antibiotics as herein defined in greatly potentiated by certain inducers that act to improve certain transport pathways in bacteria.

In accordance with the present invention the antibacterial activity of a phosphonomycin antibiotic in a non-human animal is potentiated by bringing bacteria into contact with an inducer selected from phosphatides, sugar phosphates and salts thereof, and polyhydric alcohols, whereby an existing phosphonomycin transport pathway in the bacteria is enhanced or a new such pathway is brought into being, and subsequently or simultaneously bringing the bacteria into contact with a phosphonomycin antibiotic.

This present invention also provides an antibiotic composition comprising a phosphonomycin antibiotic as herein defined, an inducer of a phosphonomycin transport system in bacteria, selected from phosphatides, sugar phosphates and salts thereof, and polyhydric alcohols, and a pharmaceutically acceptable carrier, diluent or vehicle.

Thus, compounds enhancing the activity of the α-glycerophosphate transport system, or evoking *de novo* a hexose-phosphate system in bacteria can be used either prior to or concomitantly with a phosphonomycin antibiotic to potentiate its activity, thereby permitting therapy at tissue levels obtainable by reasonable dosage. For example, microorganisms that exhibit no evidence of sensitivity to a particular antibiotic either because of the absence of a transport pathway or the presence of only low levels of suitable transport pathways are rendered sensitive to the antibiotic by exposure to an inducer of the type defined above to provide a suitable pathway or an enhancement of the existing pathway. In those cases where less than the maximal rate of synthesis of the susceptible transport system occurs prior to the introduction of the inducer to the medium, the inducer is said to enhance the rate of their synthesis and thus enhance the activity per cell of the transport system. In those cases where the rate of synthesis of a particular class is virtually zero in the absence of inducers, the latter introduce a new transport pathway into being. The inducer is frequently a substrate for one of the group of proteins it enhances or evokes.

By virtue of the activity of the inducers used in accordance with this present invention, organisms stimulated by them accumulate higher levels of phosphonomycin and are thus killed by relatively small doses of the antibiotic. Organisms that show no evidence of sensitivity toward phosphonomycin, either because the α-glycerophosphate system is present at only low levels or is absent altogether as a result of mutation to phosphonomycin resistance, are rendered sensitive to this antibiotic by the evocation of the hexose-phosphate transport pathway. Thus sensitivity is conferred on bacteria populations that are otherwise untreatable, either through prior acquisition of resistance to the antibiotic or through intrinsic insensitivity to the antibiotic.

Another advantage that may result from the present invention is that if two or more independent transport systems are inducible in a microorganism, the incidence of antibiotic resistance is much lower, since the simultaneous loss by mutation of two pathways is rare in microorganisms.

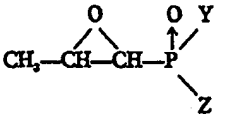
It should be emphasized that the inducers described herein are not antibiotics or antimetabolites but that they stimulate the biosynthesis of natural nutrient transport mechanisms that mediate the entry into the cell of the phosphonomycin antibiotic. This phenomenon applies uniquely to the phosphonomycin antibiotics since it has been observed that bacterial strains thus induced show no increase in sensitivity to other antibiotics tested. An important advantageous consequence of using the compounds serving as inducers is that the inducer need not be present when the transport systems mediate the entry of antibiotic into the bacterium. Thus, inducers that might compete with the phosphonomycin antibiotic for the transport proteins when simultaneously present with it and thus impair the transport pathway may be added well prior to the administration of the antibiotic and be given opportunity to dissipate within the host. In addition, the present invention does not require that the blood levels and excretion rate of two or more components be matched with each other, which is normally the case when two antibiotics are coadministered to give a synergistic effect. Therefore, the difficulty encountered in the past of establishing potent levels of two different synergizing drugs does not arise in the present invention since the inducers used need only to induce the transport pathway and then disappear; the transport proteins produced remain and thereby provide a means of entry of antibiotic into the bacteria cell. Sugar phosphates that are preferred for use in the methods and compositions of the present invention include glucose - 6 - phosphate, fructose - 6 - phosphate, mannose - 6 - phosphate, glucose - 1 - phosphate, 2 - deoxy - glucose - 6 - phosphate, 2 - amino - 2 - deoxy - glucose - 6 - phosphate, glucose - 1,6 - diphosphate, galactose - 6 - phosphate and ribose - 5 - phosphate. Thus, when susceptible bacteria are contacted with these sugar phosphates either prior to or concomitantly with a phosphonomycin antibiotic, such phosphates or an active metabolite derived therefrom potentiate the activity of the antibiotic, and it is then possible to use much smaller amounts of the antibiotic than would otherwise be necessary to control the pathogen. This observed co-action in inducing a hexose - 6 - phosphate transport system to potentiate the effectiveness of the phosphonomycin antibiotic is indeed remarkable and entirely unexpected. For example, in tests in mice against *E. coli* it is found that using a combination of glucose - 6 - phosphate and the antibiotic, the dose of phosphonomycin antibiotic needed to protect one-half the mice is less than one-tenth that required of antibiotic alone.

The coaction of the inducers described herein and the phosphonomycin antibiotic provide a valuable means for controlling and eliminating bacteria which are otherwise resistant to the action of a phosphonomycin antibiotic. Thus in accordance with the present invention a combination of the induced and the antibiotic in a suitable vehicle can be prepared by well known procedures, and used topically for the treatment of infections. Alternatively and in accordance with another embodiment of the present invention, the inducer and the antibiotic can be administered parenterally or orally to an infected non-human animal host either separately or in combination in a suitable pharmaceutical carrier, or one can be administered parenterally and the second can be given orally.

The pharmaceutical forms of the antibiotic and/or the inducing compounds, which constitute another aspect of the present invention, can be prepared in accordance with well known procedures using suitable pharmaceutical solid or liquid diluents. The compositions can be in the form of tablets, powders, granules, capsules, suspensions, solutions, elixirs, syrups or other dosage forms particularly suitable for oral administration. It can also be in the form of sterilized solutions or suspensions for parenteral administration. In such products the sterile vehicle can be a sterile solution or suspension. The compositions containing the antibiotic can be admixed with solid diluents and/or tableting adjuvants such as corn starch, talc, stearic acid, magnesium stearate and gums. The usual encapsulating or tableting materials useful in preparing pharmaceutical products can be used so long as they are not incompatible with the antibiotic or the inducing compounds. These dosage forms can contain from 25 to 500 mg of the active substances and can be administered in doses given 1 to 6 times per day depending upon the patient's age and

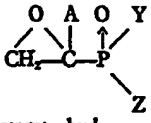
condition, the infection and the mode of administration.

The term "phosphonomycin antibiotic" as used herein includes phosphonomycin and its derivatives of the formula:



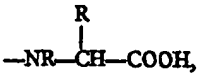
(I)

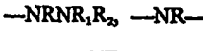
and the corresponding analogues of the formula:



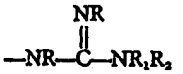
(II)

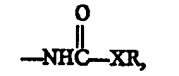
where A represents hydrogen of C_{1-6} alkyl, and each of Y and Z, which are the same or different represents OH, OR, $—NR_1R_2$,

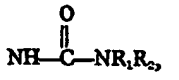




$—NROR$, $—NRNR_1R_2$, $—NR—N=CR_1R_2$,







$—N=C=X$, or $—N_2$, where R is hydrogen or a univalent substituted or unsubstituted hydrocarbon group and each of R_1 and R_2 represents hydrogen, acyl, or a univalent substituted or unsubetituted hydrocarbon group, the substituents in the substituted hydrocarbon groups in the definitions of R, R_1 and R_2 being amino, nitro, halo or oxygen-containing substituents. Also included in formulae I and II are the inorganic and organic salts of those compounds in which Y and/or Z is —OH, and the cyclic derivatives in which Y and Z are connected via a residue of a polyfunctional hydrocarbon compound such as a straight or branched-chain alkylene, aralkylene, arylene polyamine, or aminoalcohol, such as ethylenediamine, monoethanolamine, phenylenediamine, naphthalenediamine or *o*-aminophenol, and those cyclic derivatives in which $—NR_1R_2$ represents the residue of a cyclic primary or secondary amine, for example, morpholine, piperidine or pyrrolidine.

Where R, R_1 or R_2 in formulae I and II represent a univalent substituted or unsubstituted hydrocarbon radical, it can be aliphatic, cycloaliphatic, araliphatic or aromatic and can, if desired, be further substituted. When aliphatic, it can be substituted or unsubstituted alkyl, alkenyl or alkynyl. R, R_1 and R_2 can also be aralkyl or substituted aralkyl such as benzyl, phenethyl, phenylpropyl, *p*-halobenzyl or *o*-, *m*- or *p*-alkoxybenzyl, nitrobenzyl, aminophenethyl, pyridylethyl, nitrofurylmethyl or thienylpropyl, and aryl or substituted aryl, such as phenyl, naphthyl or substituted phenyl.

Thus, in accordance with the foregoing, the amide group or groups can be derived from compounds that are themselves antibacterial. Examples of such compounds that might be mentioned are 6-aminopenicillanic acid, 7-aminocephalosporanic acid, sulfa compounds such as sulfanilamide, sulfadiazine, sulfamerizine, sulfamethazine, sulfadimetine, sulfapyridine, sulfathiazole, sulfisoxazole, thiodiazole, sulfacetamide, sulfaguanidine, sulfaquinoxaline, and *p*-aminophenylsulfonamide, and *p*-aminobenzenesulfonic acid, and antibiotic agents such as ampicillin, streptomycin, dihydrostreptomycin, cycloserine, cephaloglycin and cephalixin.

The compounds of formulae I and II where at least one of Y and Z is —OH will form organic and inorganic salts, which constitute a preferred aspect of the invention because they are more stable than the free acid. Examples of such salts are inorganic metallic salts such as as the sodium, aluminium, potassium, ammonium, calcium, magnesium, silver and iron salts. Organic salts that may be mentioned as representative include the salts with primary, secondary or tertiary amines such as monoalkylamines, dialkylamines, trialkylamines and nitrogen-containing heterocyclic amines. Representative examples are salts with amines such as α-phenethylamine, diethylamine, quinine, brucine, lysine, protamine, arginine, procaine, ethanolamine, morphine, benzylamine, ethylenediamine, N,N' - dibenzylethylenediamine, diethanolamine, piperazine, dimethylaminoethanol, 2 - amino - 2 - methyl - 1 - propanol, theophylline, esters of amino acids, and N-methylglucamine. If desired, the basic residue of the salt may be a biologically active amine such as erythromycin, oleandomycin or novobiocin.

The monoamide-monoester derivatives and particularly those compounds having a labile ester substituent are especially valuable derivatives. By the term "labile ester" is meant a group which is readily hydrolyzed biologically, for example by enzymes in the body fluids of animals including man, to produce the free acid or a salt thereof which is more active as an antibiotic agent. The amide or substituted amide groups present in the amide-ester derivatives are also readily hydrolysed biologically in the body fluids and hence the

amide-labile ester derivatives are useful in antibiotic therapy.

Suitable labile ester groups include ethers of the formula $-CH_2OR$, a phenacyloxymethyl group, acyloxy methyl groups of the formula $-CH_2OA$ where A is an acyl group comprising an organic radical derived from an organic acid by the removal of the hydroxy group, amide and substituted amide derivatives of such acyloxymethyl substituents, acylaminomethyl groups of the formula $-CH_2NHA$ where A is the same as defined above, thiomethyl ethers of the formula $-CH_2SR$, an ethynyloxy group of the formula $-CH_2OC{\equiv}CH$, substituted ethynyloxy groups of the formula $-CH_2OC{\equiv}CR$, a vinyloxymethyl group of the formula $-CH_2OCH{=}CH_2$, substituted vinyloxymethyl groups of the formulae $-CH_2OCH{=}CHR$ or $-CH_2OCH{=}CRR$, or a nitro oxy group of the formula $-CH_2ONO_2$. R in each of the foregoing formulae is a univalent substituted or unsubstituted hydrocarbon group.

Specific examples of such labile ester groups that might be mentioned are methoxymethyl, tetrahydropyranyloxymethyl, phenacyloxymethyl, acetoxymethyl, butyryloxymethyl, isobutyryloxymethyl, pivaloyloxymethyl, benzoyloxymethyl, 2 - methylbenzoyloxymethyl, 2,6 - dimethylbenzoyloxymethyl, 2 - methyl - 6 - chlorobenzoyloxymethyl, 3 - trifluoromethylbenzoyloxymethyl, 2 - nitrobenzoyloxymethyl, 2 - methylthiobenzoyloxymethyl, 2 - thienylcarbonyloxymethyl, 2 - furylcarbonyloxymethyl, 3 - pyridylcarbonyloxymethyl, pyrazinylcarbonyloxymethyl, 2 - methylcyclopentylcarbonyloxymethyl, 1 - adamantylcarbonyloxymethyl, phenylsulfonylmethyl, phosphonooxymethyl, diethylphosphonoxymethyl, carbethoxyoxymethyl, carbamoyloxymethyl, N - methylcarbamoyloxymethyl, N,N - dimethylcarbamoyloxymethyl, phenylsulfamoyloxymethyl, acetamimmethyl, benzoylaminomethyl, methylthiomethyl, phenylthiomethyl, vinyloxymethyl, 1 - methylvinyloxymethyl, and nitrooxymethyl.

The following examples illustrate embodiments of the invention. 'Difco' and 'Dowex' are trademarks.

Example 1

Effect of Combining Glycerol or DL-α-Glycerophosphate with Phosphonomycin on its Inhibition of Several Strains of Bacteria

Overnight cultures of the indicated strains in Nutrient Broth (Difco) were diluted one hundred fold, and 0.05 ml. portion was swabbed over the surface of a 2mm-deep layer of the indicated solid growth media in 50cm² petri dishes. Sensitivity discs, consisting of a 7mm-diameter filter-paper circle containing either 5 or 30 μg of phosphonomycin with an additional amount of glycerol or disodium DL-α-glycerophosphate were placed on the surface of the seeded agar. Zones of inhibition were measured after 18 hours of incubation at 37°C. The results are shown in the following table:

Strain	Medium	µg P	Zone Size mm Diameter				
			P	A	B	C	D
E. coli MB 2489	Nutrient Agar	5	11	45	40	10	0
E. coli MB 2498	Nutrient Agar	30	13	13	12	13	0
E. coli MB 2489 A2	Nutrient Agar	5	8	47	43	10	0
E. coli MB 2017	Nutrient Agar	5	12	29	26	16	0
Pseudo. aeruginosa T 9	Nutrient Agar	5	15	40	18	15	0
Pseudo. aeruginosa T 19	Nutrient Agar	30	18	31	29	16	0
Pr. mirabilis T 10	Mueller Hinton Agar	5	21	25	23	23	23
D. pneumoniae I 37	Nutrient Agar + 10% Horse serum	30	10	15	—	16	—
D. pneumoniae I 37	Brain Heart Infusion + 10% Horse serum	30	12	14	—	15	—
D. pneumoniae I 2483	Brain Heart Infusion + 10% Horse serum	30	10	13	—	14	—
Strep. pyogenes 3009	Brain Heart Infusion + 10% Horse serum	30	15	19	—	18	0
Strep. pyogenes 1685	Brain Heart Infusion + 10% Horse serum	30	14	17	—	13	0
Sal. schott-muelleri 1814	Brain Heart Infusion	30	11	19	—	12	—
Sal. typhi-murium MB 1995	Brain Heart Infusion	30	15	19	—	15	—
Sal. typhosa. 2866	Brain Heart Infusion	30	19	22	—	19	—

Key to identity and amount of potentiator added to disc in combination with phosphonomycin;

P — Phosphonomycin (disodium salt) (amount indicated in the column to the left).

A — Glycerol, 10 mg.

B — Glycerol, 1 mg.

C — DL-α-glycerophosphate, disodium, 10 µg.

D — DL-α-glycerophosphate, disodium, 100 µg.

Glycerol is seen to affect a broad spectrum of strains, failing only in the case of *E. coli 2498* (a mutant derivative of *MB 2489*) which is known to lack α-glycerophosphate transport activity, and *Proteus mirabilis* (T 10). The latter strain, in common with all sensitive *Proteus* species examined, has a very active α-glycerophosphate transport system, which is in all probability not subject to enhancement by inducers.

Few significant examples of enhanced sensitivity are observed at the low level of added disodium *DL*-α-glycerophosphate, even though it is a known inducer of the transport system at least in *MB 2489*. Rather, antagonism is demonstrable at the high level (100 μg) added to the disc. This phenomenon probably represents the expected competition between phosphonomycin and α-glycerophosphate for their common transport system. In contrast, glycerol, while an *inducer*, is not a *substrate*, and therefore does not preoccupy the transport system whose activity it has stimulated.

Example 2

The Effect of Glucose-6-Phosphate on the Sensitivity of *Escherichia coli* and *Staphylococcus aureus* to *Phosphonomycin in Liquid Media of Various Composition*

Overnight broth cultures were diluted 1: 10,000 (10^7 cells/ml) in the indicated media and combined with an equal volume of media containing various levels of disodium phosphonomycin. The minimal inhibitory concentration (M.I.C.) was that final concentration of phosphonomycin below which turbidity was observed following a 24-hour incubation at 35°C.

Medium	*M.I.C. μg/ml* *Staphylococcus aureus* MB 2949	*Phosphonomycin* *Escherichia coli* MB 2017
Mueller Hinton Broth (Difco)	50	3.12
Mueller Hinton Broth plus disodium glucose-6-phosphate, 25 μg/ml	3.12	0.78
Nutrient Broth (Difco)	25	12.5
Nutrient Broth (Difco) plus disodium glucose-6-phosphate	1.5	0.39
Nutrient Broth (Difco) plus 5% v/v Defibrinated Sheep Blood (Gibco)	3.12	0.39

With both media, glucose-6-phosphate is observed to potentiate by a factor of 4 to 40 the sensitivity of Gram-positive and Gram-negative pathogens. In Nutrient Broth, the effect observed with glucose-6-phosphate mimics that observed with sheep blood.

Example 3

The Influence of Glucose-6-Phosphate on the Fraction of Bacterial populations that Survive a Given Level of Phosphonomycin

Various dilutions of overnight broth cultures of the indicated bacterial strains were swabbed over the surface of petri dishes containing Mueller Hinton medium, 1.5% Agar (Difco), and supplemented with the indicated levels of disodium phosphonomycin, with or without 25 μg/ml of disodium glucose-6-phosphate. From the number of colonies present at a particular dilution of input organisms, the number of input cells surviving a given level of phosphonomycin with and without glucose-6-phosphate are calculated and shown in the following table:

Strain	µg/ml Phosphonomycin	Number of surviving colony formers per ml: Mueller Hinton Agar alone	Mueller Hinton Agar plus 25 µg/ml glucose-6-phosphate
Escherichia coli MB 2017	0	3×10^9	3×10^9
	10	3×10^5	5×10^8
	30	3×10^5	50
	100	3×10^5	<10
Staphylococcus aureus MB 2949	0	3×10^9	3×10^9
	10	3×10^8	3×10^5
	30	3×10^6	<100
	100	3×10^8	<10
Aerobacter aerogenes MB 3287	0	7×10^9	5×10^9
	10	3×10^7	1×10^5
	30	5×10^5	5×10^4
	100	3×10^5	5×10^2
Staphylococcus aureus MB 3036	0	2×10^8	2×10^8
	10	2×10^5	1×10^3
	30	2×10^4	1×10^3
	100	<10	<10
Shigella sp. MB 3298	0	2×10^8	2×10^8
	10	3×10^7	1×10^8
	30	3×10^7	1×10^3
	100	1×10^4	6×10^2

In all cases, a smaller proportion of the input bacterial population survives to form colonies on the plate containing glucose-6-phosphates than on the plate that lacks this potentiation. In most cases, the substantial residual population (of the order of 1 in 10^3 to 1 in 10^4) that survive high levels of phosphonomycin are eradicated or much reduced when glucose-6-phosphate is also present. Thus a sensitization of the bulk population and an elimination of resisters are evident when this inducer is present.

Example 4

Effect of Glucose-6-Phosphate on the Size of the Zone of Inhibition Surrounding Sensitivity Discs Containing this Sugar Phosphate in Combination with Phosphonomycin

Overnight cultures of the indicated strains grown in Nutrient Broth (Difco) were diluted one hundred fold, and a 0.05-ml portion was swabbed over the surface of a petri dish containing 10 ml of Mueller Hinton Agar (Difco). Sensitivity discs, consisting of a 7 mm diameter filter-paper disc containing either 5 or 30 µg of disodium phosphonomycin with or without an additional 5 µg of disodium glucose-6-phosphate, were placed on the surface of the seeded agar. Zones of inhibition were measured after 18 hours of incubation at 37°C.

Bacterial strain	Diameter of Zone of Inhibition mm			
	5 μg — 30 μg Phosphonomycin without glucose-6-phosphate		5 μg — 30 μg Phosphonomycin plus glucose-6-phosphate	
Escherichia coli MB 2017	11	16	20	24
Staphylococcus aureus MB 2949	0	11	13	20
Aerobacter aerogenes MB 3287	0	0	16	26
Staphylococcus aureus MB 3036	0	30	27	40
Shigella sp. MB 3298	0	10	24	37

The sensitization of cells by glucose-6-phosphate noted in the prior example is here made evident by substantial increases in the zone of inhibition surrounding discs that contain a mixture of phosphonomycin and glucose-6-phosphate. It is further noteworthy that in all cases where zone enhancement is observed in the presence of glucose-6-phosphate, the inhibited area is found to be relatively free of the myriad of drug resistant colonies that surround a disc of phosphonomycin by itself. These observations are consistent with the induction by glucose-6-phosphate of an alternate pathway for the entry of phosphonomycin into cells that have lost their normally expressed α-glycerophosphate transport pathway.

Example 5

Example of a Method for Screening Phosphate Esters as Inducers of Latent Transport Systems of Phosphonomycin in *Escherichia coli*

The strain of *Escherichia coli* MB 2498 is a subculture of mutant 6 described in Table 1 of the Journal of Molecular Biology, *31*, 371 (1968). It lacks the ability to grow on or accumulate *L*-α-glycerophosphate, and is resistant to levels of phosphonomycin up to 70 μg/ml in Nutrient Broth. (The parent wild type strain is completely inhibited by 10 μg/ml of disodium phosphonomycin.) MB 2498 is also lacking in alkaline phosphatase activity, and therefore degrades exogenous phosphate esters to a minimum extent.

In a search for inducers of additional transport systems for phosphonomycin, 0.05 ml of a 10^7 cell/ml suspension was smeared over the surface of a 50 cm^2 petri plate containing 10 ml Nutrient Broth, 1.5% Agar (Difco), and 25 μg/ml of disodium phosphonomycin. Paper discs measuring 7 mm in diameter and capable of absorbing 0.25 ml of solvent were treated with solutions of various phosphate esters and applied to the agar surface. Zones of inhibition were measured after 18 hours incubation at 37°C.

Compound tested	μg present in disc	Zone of inhibition mm diameter
None	—	0
Glucose-6-phosphate disodium	1.0	31
	0.5	27
	0.1	18
Fructose-6-phosphate disodium	10.0	42
Mannose-6-phosphate disodium	6.0	31
2-deoxy-glucose-6-phosphate disodium	1.0	27
2-amino-2-deoxy-glucose-6-phosphate disodium	25.0	34
Ribose-5-phosphate disodium, monohydrate	25	35
Phosphatidyl ethanolamine	30	29
Glucose-1',6'-diphosphate, tetrapotassium pentahydrate	25	34
Glucose-1-phosphate disodium	25	31
5-phosphoryl ribose-1-pyrophosphate, dimagnesium dihydrate	25	25
Riboflavin-5-phosphate disodium	25	14

Among the compounds in the above test showing no activity at a level of 25 μg per disc were: inositolphosphate, adenosine - 5' - phosphate, galactose - 1 - phosphate, 2'-deoxy ribose - 1' - phosphate, α - *D* - ribose - 1 - phosphate, β - *D* - ribose - 1 - phosphate, α - *D* - xylopyranose - 1 - phosphate, gluconic-6 - phosphate, mannose - 1 - phosphate, erythrose - 4 - phosphate, pyridoxine - phosphate, thiamine monophosphate, *D* - galactose - 6 - phosphate, *D* - fructose - 1 - phosphate, fructose - 1 - 6 - diphosphate, phosphoserine, phosphatidyl choline and N,N - dimethyl - *L* - phosphatidyl ethanolamine, as well as an extensive list of non-phosphorylated tetroses, pentoses, and hexoses. Thus the potentiation phenomenon shows a degree of specificity, which in the case of the hexose phosphates seems to include primarily those compounds that are generated by the hexokinases and includes those hexose phosphates known to induce the glucose - 6 - phosphate transport system (Compounds 1, 2, 3, 4, and 9).

None of the potentiating compounds at the levels tested produced zones of inhibition with MB 2498 seeded plates consisting of Nutrient Broth/Agar lacking MK 955.

Example 6

Effect of Combining Various Phosphate Esters on the Inhibition of Several Strains of Bacteria

Overnight cultures of the indicated strains in Nutrient Broth (Difco) were diluted one hundred fold, and a 0.05-ml portion was swabbed over the surface of a 2 mm deep layer of the indicated solid growth media. Sensitivity discs, consisting of a 7-mm-diameter filter-paper circular disc containing either 5 or 30 μg of disodium phosphonomycin with an additional amount of the indicated phosphate esters, were placed on the surface of the seeded agar. Zones of inhibition were measured after 18 hours of incubation at 37°C.

Strain	P µg	Zone size mm diameter (see key below for phosphate ester added) P	A	B	C	D	E	F	G	H	I
MB 2489	5	11	25	25	20	30	22	21	18	15	22
MB 2489 A2	5	8	10	10	10	10	21	10	11	10	10
MB 2498	30	13	33	33	26	24	21	31	26	21	30
MB 24980	30	11	12	10	10	12	14	10	11	11	12
MB 2017	5	12	23	22	20	17	16	21	19	17	19
T 14	5	0	13	15	0	0	0	12	0	0	0
T 27	30	0	20	20	0	0	01	18	13	0	14
T 9	5	15	16	15	15	15	12	15	16	16	16
T 10	5	21	23	23	23	23	22	22	22	23	23
T 19	30	18	17	17	17	15	18	15	17	17	15

Key to the identity and amount of phosphate esters added to disc together with phosphonomycin.

P — Phosphonomycin disodium salt (amount indicated for the column to the left).

A — glucose-6-phosphate 5 µg.

B — 2'-deoxy-glucose-6-phosphate 5 µg.

C — ribose-5-phosphate 25 µg.

D — phosphatidyl ethanolamine 25 µg.

E — riboflavin-5-phosphate 50 µg.

F — fructose-6-phosphate 5 µg.

G — mannose-6-phosphate 5 µg.

H — 2-amino-2-deoxy-glucose-6-phosphate 25 µg.

I — glucose-1-phosphate 25 µg.

Strain *MB 2489* is an *Escherichia coli* that grows well on α-glycerophosphate and D-glucose-6-phosphate. It exhibits on Nutrient Broth Agar moderate sensitivity to phosphonomycin that is much enhanced by the whole series of hexose-phosphate esters (A, B, F, G, I) that have been found capable of inducing glucose-6-phosphate transport pathway.

Strain *MB 2489 A2* was isolated from the periphery of the enhanced zone of inhibition surrounding a disc bearing phosphonomycin (5 µg) and glucose-6-phosphate (25 µg). It was found to grow well on α-glycerophosphate, but to show no stimulation of growth by glucose-6-phosphate. Although this strain is as sensitive to phosphonomycin alone as is the parent *MB2489* (as expected, since its α-glycerophosphate transport system is active), it fails to be stimulated on Nutrient Broth Agar by any of the hexose phosphate inducers of the glucose-6-phosphate transport system. In addition, the failure of ribose-5-phosphate and phosphatidyl ethanolamine to synergise suggests that these esters also induce the glucose-6-phosphate transport system. However, the activity still exhibited by riboflavin-5-phosphate implies the presence of yet a third inducible pathway that mediates enhanced phosphonomycin transport.

Strain *MB 2498* is a mutant of *MB 2489* that lacks the α-glycerophosphate pathway (i.e. it fails to grow on α-glycerophosphate but

retains the glucose-6-phosphate inducible system). Although it is much less sensitive on Nutrient Broth Agar to phosphonomycin as such than *MB 2489*, it retains the ability to be stimulated by the phosphate ester inducers.

Strain *MB 24980* was isolated as a resistant colony from a plate containing 25 μg/ml of phosphonomycin and 25 μg/ml of glucose-6-phosphate. In keeping with the above results, its sensitivity on Nutrient Broth Agar to phosphonomycin is enhanced solely by riboflavin-5-phosphate.

Strain *MB 2017* is an *Escherichia coli* pathogenic for mice. It exhibits broad sensitization on Nutrient Broth Agar by the entire class of phosphate ester inducers.

Strain *T 14* is a *Klebsiella* species isolated from the urine of a patient just about to receive phosphonomycin therapy; *T 27* is a *Klebsiella* species isolated from the urine of a patient who had been on phosphonomycin oral therapy for seven days. Both of the strains are resistant on Nutrient Broth Agar to phosphonomycin by itself, but show moderate sensitivity in the presence of a variety of inducers of the glucose-6-phosphate pathway.

Strains *T 9 and T 19* are strains of *Pseudomonas aeruginosa* isolated from the urine of infected humans. They show no significant response on Nutrient Broth Agar to the above phosphate esters.

Strain *T 10* is a *Proteus mirabilis* strain isolated from the urine of an infected human, and shows no significant response on Mueller Hinton Agar to any of the above phosphate esters.

Example 7

The Effect seen in Various Growth Media of Glucose-6-Phosphate on the Size of the Zone of Inhibition Surrounding Sensitivity Discs Containing this Sugar Phosphate in Combination with Phosphonomycin

An overnight culture of *Escherichia coli, MB 2489*, grown in Nutrient Broth was diluted one hundred fold, and an aliquot of 0.05 ml was swabbed over the surface of a 2 mm deep agar medium consisting of either Nutrient Broth, 1.5% Agar (Difco), Brain Heart Infusion, 1.5% Agar (Difco), Mueller Hinton Agar (Difco), Trypticase-Soy Agar (BBL), or a "human urine-agar." The latter medium was prepared by centrifuging adult male urine collected immediately after sleep, membrane filtering the supernatant to achieve sterility and combining the filtrate with one tenth volume of autoclaved 15% Noble Agar (Difco) in water to produce a solid medium. Sensitivity discs, consisting of a 7mm diameter filter paper disc containing either 5 or 30 μg of disodium phosphonomycin with and without disodium glucose-6-phosphate, were placed on the surface of the seeded agar. Zones of inhibition were measured after 18 hours of incubation at 37°C.

	Diameter of Zone of Inhibition — mm			
	5 μg	30 μg	5 μg	30 μg
Medium used	Phosphonomycin alone		Phosphonomycin plus glucose-6-phosphate (25 μg)	
Nutrient Broth	12	24	28	33
Mueller Hinton Broth	0	14	20	26
Brain Heart Infusion	0	12	16	20
Trypticase-Soy Broth	0	15	18	24
Human urine	9	19	14	26

The activity of phosphonomycin alone is clearly antagonized relative to Nutrient Broth in the other media employed. This antagonism can be attributed to a major extent to high levels of sodium chloride in Mueller Hinton, glucose and phosphate in Brain Heart Infusion and Trypticase-Soy and phosphate ion in human urine. These interfering phenomena are substantially overcome by the inclusion of glucose-6-phosphate in the sensitivity disc.

Example 8

Effect of Glucose-6-Phosphate on the Sensitivity of *Escherichia Coli* Strains to Several Phosphonomycin Analogues

Overnight cultures of *Escherichia coli*, strains MB 2489 (possessing both the α-glycerophosphate transport and the glucose-6-phosphate transport systems) and MB 2498 (possessing only the glucose-6-phosphate pathway, and therefore relatively resistant to

phosphonomycin alone) were diluted one hundred fold and a 0.05-ml portion was swabbed over the surface of a 2 mm of Nutrient Broth, 1.5% agar (Difco). Sensitivity discs consisting of a 7-mm-diameter paper disc containing disodium phosphonomycin or one of the indicated analogues in the stated quantities together with an additional 5 µg of disodium glucose-6-phosphate where indicated, were placed on the surface of the seeded agar. Zones of inhibition were measured after 18 hours of incubation at 37°C.

Active substance	Amount µg	MB 2489 no G—6—P	MB 2489 + G—6—P	MB 2498 no G—6—P	MB 2498 + G—6—P
Phosphonomycin	5	14	28	0	30
	2.5	12	25	0	24
	1.0	0	20	0	24
	0.3	0	18	0	15
1-methyl-1,2 epoxyethyl phosphonic acid, mono dicyclohexyl amine salts	500	20	38	9	42
	50	0	32	0	36
	5	0	13	0	16
1,2 epoxyethyl phosphonic acid, dicyclohexyl ammonium salt	500	16	35	8	38
	50	0	26	0	29
	5	0	10	0	12

Glucose-6-phosphate is observed to so potentiate the sensitivity of phosphonomycin-sensitive and resistant strains that they now respond to weak analogues of phosphonomycin to the same degree as to phosphonomycin by itself.

Example 9

Effect of Phosphonomycin and Glucose-6-Phosphate and Combinations thereof in Treatment of Infected Mice

Female C.D.1 mice of average weight, 22.5 g., were infected intraperitoneally with 16-hour broth cultures appropriately diluted in brain heart infusion. For *E. coli* the challenge contained 2.5 x 10^7 cells or 7 LD_{50} doses; for *Shigella*, 2.3 x 10^8 cells or 3 LD_{50} doses. At the time of infection the disodium salt of phosphonomycin and sodium glucose-6-phosphate was administered separately in 0.25 ml. subcutaneously at a separate site, one on each side of the dorsal surface. The results of these tests are shown in the following table:

Test Organism	G—6—P µg	ED_{50} subcutaneously: Disodium phosphonomycin (DSP) µg	DSP µg	DSP %	DSP + 1.0 mg G—6—P µg	DSP + 1.0 mg G—6—P %	DSP + 0.1 mg G—6—P µg	DSP + 0.1 mg G—6—P %
Escherichia coli 2017	>4000	155	100	12	8	91	58	
Shigella (118—57) 3303	>4000	1000	100	82	8	1500	150	

Example 10

Effect of Phosphonomycin and Glucose-6-Phosphate, and Combinations thereof in the Treatment of Infected Mice

In further mouse tests carried out as described in Example I except that the antibiotic was combined with the sodium glucose-6-phosphate and given in one injection, the following results were obtained in mice infected with *Aerobacter aerogenes* and *Staphylococcus aureus*:

Test Organism	Disodium phosphonomycin (DSP) s.c. ED_{50} in μg: DSP Alone	μg G—6—P added to DSP: 4000	1000	500	100	G—6—P ED_{50} used alone (μg)
Aerobacter aerogenes 3148	10,000+	287	3,000	7,700	10,000	>4,000
Staphylococcus aureus Smith 2949	96	22	50			>4,000

Example 11

Effect of Fructose-6-Phosphate in Potentiating Phosphonomycin in Mice

The efficacy of fructose-6-phosphate in potentiating the control by phosphonomycin of experimental bacterial infections in mice was compared with that of glucose-6-phosphate in tests following the protocol of Example 10. Again the antibiotic was combined with the sugar phosphate in a single subcutaneous injection administered at the time of intraperitoneal inoculation with *Escherichia coli, MB 2017.*

Potentiator	Dose (μg)	ED_{50}, μg Phosphonomycin
None	—	2000
Glucose-6-phosphate disodium	1000	17
	600	31
	300	69
	100	470
Fructose-6-phosphate disodium	1000	17
	600	57
	300	202
	100	534

Fructose-6-phosphate is seen to exercise a degree of potentiation toward phosphonomycin that is comparable with that observed previously in the parallel experiment with glucose-6-phosphate. This equivalency was expected both from the similar enhancement of inhibition observed *in vitro*, in Example 5, when either of these sugar phosphates were combined with phosphonomycin, and from the certainty of their interconversion by the ample phosphoglucoseisomerase activity present in plasma.

Example 12

Therapeutic Efficacy of Phosphonomycin Administered Orally to Infected Mice Receiving Glucose-6-Phosphate by Either the Oral or Subcutaneous Route

The protocol of Example 9 was followed for the case of *Escherichia coli* 2017 except that immediately upon infection the disodium salt of phosphonomycin was administered *orally*, while disodium glucose-6-phosphate, where indicated, was administered either orally or by the subcutaneous route. In no case was protection observed when glucose-6-phosphate was administered alone at the 4000 μg level, orally or subcutaneously, in the *absence* of phosphonomycin.

Glucose-6-phosphate μg	Route	Dose of phosphonomycin (μg) administered orally that protects 50% of animals (ED_{50})
0	—	2000
1000	orally	2000
100	subcutaneous	37

Glucose-6-phosphate is an effective potentiator of therapy for phosphonomycin administered orally (25 fold sensitization) when sugar phosphate is administered subcutaneously. No sensitization is observed when the sugar phosphate is administered orally at that level.

Example 13

Therapeutic Efficacy of Phosphonomycin Administered Parenterally to Infected Mice Receiving Glucose-6-Phosphate Salts Orally

The protocol of Example 9 was followed for the case of *Escherichia coli* 2017 except that immediately after infection the disodium salt of phosphonomycin was administered subcutaneously, while glucose - 6 - phosphate in the indicated form was administered orally by gavage in 0.25 ml. of water. In no case was protection observed with the glucose - 6 - phosphate salts administered alone, nor did the oral administration solely of 2.5 mg. of *n*-octylammonium chloride (without glucose - 6-phosphate) decrease the ED_{50} of phosphonomycin coadministered parenterally. The *n*-octyl - ammonium salts of glucose - 6 - phosphate were prepared by converting its disodium salt to the free acid by passage through a column containing a 20-fold excess of Dowex-50 (H^+ form), followed by neutralisation of portions of the eluate with either 0.7, 1.5, or 2.0 molar equivalents of the free *n*-octylamine base, and 1.3, 0.5, or 0 molar equivalents of NaOH respectively, to give a final pH of 7.5 in each case.

Glucose-6-phosphate salt (mg)		Dose of phosphonomycin that protects 50% of animals (ED_{50}) (μg)
TEST I		
none		500
disodium salt	100	27
	50	63
	25	125
	12.5	302
	6.25	531
sodium 0.5 + *n*-octyl-ammonium 1.5	10	66
	1	302
TEST II		
none		827
di-*n*-octyl-ammonium	5	125
sodium 0.5 *n*-octyl-ammonium 1.5	5	168
sodium 1.3 *n*-octyl-ammonium 0.7	5	714

Glucose - 6 - phosphate administered orally potentiates therapy by phosphonomycin, and is rendered more efficient in this effect in proportion to the fraction of inorganic counterion replaced by lipophilic amine.

Example 14

Potentiation by Coadministered Galactose-6-phosphate of Phosphonomycin Therapy in Mice Infected with *Staphylococci*

An examination of the efficacy of galactose-6 - phosphate in potentiating the control by phosphonomycin of experimental *Staphylococcal* infections in mice was justified by the finding, made in an application to this strain of the methodology described in Example 6, that 25 μg of this sugar phosphate when added to a sensitivity disc bearing 5 μg of phosphonomycin produced a 21 mm zone of inhibition as opposed to a 17 mm zone for the unsupplemented disc, when the discs were placed on a Nutrient Agar plate seeded with *Staphylococcus aureus* Smith 2949. In the therapy trial below, the antibiotic was combined with the sugar phosphate in a single subcutaneous injection administered at the time of intraperitoneal injection with 10^6 cells per mouse (14 LD_{50}'s), with cells grown for 16 hours in brain-heart infusion.

Potentiator	Dose (μg)	ED_{50} (μg Phosphonomycin)
none	—	212
disodium glucose-6-phosphate	4000	91
disodium galactose-6-phosphate	4000	25

The effectiveness of galactose - 6 - phosphate as a potentiator is explained by the demonstrated existence in *Staphylococcus* of an inducible galactose - 6 - phosphate transport system. Galactose - 6 - phosphate is a metabolite of lactose hydrolysis unique to certain Gram-positive organisms, not however generated or utilized by *E. coli*. This accounts for the failure of galactose - 6 - phosphate to potentiate phosphonomycin action on *E. coli* (Example 5).

Example 15

Effect of Mannose-6-phosphate in Potentiating Phosphonomycin in Mice

The efficacy of mannose - 6 - phosphate in potentiating the control by phosphonomycin of experimental infections in mice was compared with that of glucose - 6 - phosphate, in tests following the protocol of Example 10. Again the antibiotic was titrated in combination with a series of fixed levels of sugar phosphates in a single subcutaneous injection administered at the time of intraperitoneal inoculation with *Escherichia coli* MB 2017.

Potentiator	Dose (μg)	ED_{50} (μg Phosphonomycin)
none	—	1420
disodium glucose-6-phosphate	1000	18
disodium mannose-6-phosphate	1000	19
"	500	23
"	250	92
"	125	490

Mannose - 6 - phosphate exercises a degree of potentiation toward phosphonomycin equivalent to that of comparable levels of glucose - 6 - phosphate, even though its effect *in vitro* is only one-tenth that of glucose - 6-phosphate. This discrepancy can be attributed to the conversion of mannose - 6 - phosphate to glucose - 6 - phosphate *in vivo* by sequential action of mannose - phosphate - isomerase and phosphoglucose isomerase, enzymes whose activities are demonstrable in plasma and in the walls of blood vessels.

Example 16

Potentiation by Glucose - 1 - phosphate and Ribose - 5 - phosphate of Phosphonomycin Therapy in Mice

The efficacy of glucose - 1 - phosphate and ribose - 5 - phosphate in potentiating the control by phosphonomycin of experimental bacterial infections in mice was compared with that of glucose - 6 - phosphate in tests following the protocol of Example 10. Again the antibiotic was titrated for its curative efficacy in combination with the indicated fixed levels of sugar phosphate in a single injection administered at the time of intraperitoneal inoculation with *E. coli* MB 2017.

Potentiator	Dose (μg)	ED_{50} (μg Phosphonomycin)
none	—	940
disodium glucose-6-phosphate	1000	5
dipotassium glucose-1-phosphate	1000	9
disodium ribose-5-phosphate*	1000	158

* This sample was demonstrated by a specific assay with glucose-6-phosphate dehydrogenase to be contaminated by no more than one part per thousand of glucose-6 phosphate.

The potentiating ability of 1000 μg of ribose - 5 - phosphate, while significant, is equivalent only to that produced by approximately 100 μg of glucose - 6 - phosphate (see Example 11). This degree of relative potency was anticipated from the ratio of the weights of ribose - 5 - phosphate to glucose - 6 - phosphate which produce equivalently enhanced zones of inhibition *in vitro* (Example 5). The equivalent potencies of glucose - 1 - phosphate and glucose - 6 - phosphate *in vivo*, despite differences *in vitro*, is most likely attributable to rapid conversion of the 1 - phosphate to the 6-phosphate by the action of phosphoglucomutase, known to be present in plasma.

Example 17

Potentiation by Coadministered Lactose of Phosphonomycin Therapy in Mice Infected with Streptococci

On applying the methodology described in Example 6 to the *Streptococci*, it was found that 250 μg of lactose added to a sensitivity disc bearing 30 μg of phosphonomycin produced a 27 mm diameter zone of inhibition, as compared with a 12 mm zone with an unsupplemented disc, when the discs were placed on a Nutrient Agar plate seeded with *Streptococcus faecalis* R. In the therapy trials below, 14 colony - forming units (7 LD_{50}'s) of the pathogenic *Streptococcus pyogenes* (1934) grown in brain-heart broth supplemented with 10% horse serum, were inoculated intraperitoneally. Simultaneously, 0.5 ml of either a lactose solution or a saline control were injected subcutaneously followed in Trial I by a single 0.5 ml dose of phosphonomycin orally (by gavage), and in Trial II by 4 successive 0.5 ml oral doses of antibiotic at 0, 2, 4, and 6 hours post infection.

Potentiator	Dose (mg)	ED_{50} (total phosphonomycin administered — μg)
	TRIAL I	
none	—	3,950
lactose	4	1,530
	TRIAL II	
none	—	2,100
lactose	4	800

Neutral saccharides are thus capable of potentiating phosphonomycin action *in vivo* as *in vitro*. Certain *Streptococci*, in common with *Staphylococci*, also show inducible metabolism of lactose to galactose - 6 - phosphate.

The following experiments, though not part of the invention give further background invention give further background information concerning transport systems in bacteria.

Experiment 1

Efficacy of Phosphonomycin in Protecting Mice Infected by Mutant Bacterial Isolates Displaying High Levels of the L - α - Glycerophosphate Transport System In the Absence of Inducer

Mutants were isolated from *E. coli* 2017 using a mutagenesis and mutant detection screen described in Biochimica et Biophysica Acta, Volume 60, p. 422—424, 1962, and these mutants showed high levels of α-glycerophosphate and glycerol metabolism without the need for prior growth in the presence of these inducers, such as is shown by the natural strains. The diameter of the zones of inhibition around sensitivity discs bearing 5 μg of phosphonomycin, placed on Nutrient Agar plates seeded with mutants C_1, C_2, and the parent strain, were 20, 24, and 12 mm respectively. Since we have shown (Example I), that the addition of glycerol to such discs on the parent strain increases the zone size to 26—29 mm, we conclude that the mutant strains possess levels of the phosphonomycin transport system (i.e., the *L* - α - Glycerophosphate transport system) comparable to those of induced wild type strains. Therefore, the response of these mutants to phosphonomycin therapy should be predictive of the response of induced wild type strains in other situations. Mutants C_1 and C_2 and the parent strain were grown under identical conditions (described in Example 9) and were inoculated intraperitoneally into mice at the indicated challenge levels. Mice were injected subcutaneously with phosphonomycin immediately following infection.

Bacterial Strain	No. of Cells Inoculated (virulence)	ED_{50} (μg phosphonomycin)
E. coli 2017 (parent strain)	4.2×10^8 (10 LD_{50}'s)	943
Mutant C_1	4.7×10^8 (30 LD_{50}'s)	12
Mutant C_2	1.2×10^7 (9 LD_{50}'s)	14

Thus, even though these mutants possess full virulence, they are controlled by remarkably low levels of phosphonomycin, implying that the uninduced wild-type strain displays *in vivo* far less than its full inducible capacity for responding to phosphonomycin.

Experiment 2

Effect of Coadministered Glucose - 6 - Phosphate on the Susceptibility to Phosphonomycin, *In Vitro* and *In Vivo* of a Becterial Variant That Had Acquired Resistance to Phosphonomycin During Therapy in Man.

The strains of *Escherichia coli* used below represent, in the case of M 13, an isolate from the urine of an infected female just prior to her treatment with phosphonomycin, and in the case of M 21, an isolate of the drug-resistant organisms present in the urine of this individual after therapy for 7 days with the antibiotic. The *in vitro* susceptibility tests were performed in the manner described in Example 6. The *in vivo* mouse protection trial was carried out as described in Example 9 following intraperitoneal challenge with the indicated number of organisms.

IN VITRO SUSCEPTIBILITY TESTS

	Zones of inhibition (mm) surrounding discs bearing 30 µg of phosphonomycin alone, or in combination with 5 µg of glucose-6-phosphate	
	Phosphonomycin alone	plus glucose -6-phosphate
E. coli M 13	19	28
E. coli M 21	0 (less than 7 mm)	20

CURATIVE EFFICACY OF PHOSPHONOMYCIN IN INFECTED MICE

(Ed_{50}'s in mg)

	Phosphonomycin above	Phosphonomycin co-administered with 1 mg glucose-6-phosphate, disodium
E. coli M 13		
3.7×10^7 cells	0.25	0.035
= 8 LD_{50}'s		
E. coli M 21		
1.2×10^7 cells	17.5*	2.5
= 10 LD_{50}'s		

* At the highest drug level administered to this group, 20 mg per mouse, only 3 of the 5 infected animals were protected. In the other three groups complete protection was observed at no higher than twice the median level quoted.

Since the resistant strain retained the ability to respond to phosphonomycin upon co-addition of glucose - 6 - phosphate, it may be inferred that the hexose - 6 - phosphate transport pathway is not significantly induced by endogeneous substances in the natural urinary infections of man. When transport is evoked by the intentional coadministration of inducers, resistance to phosphonomycin should be prevented or overcome, and the therapeutic elimination of such strains should be made possible.

Experiment 3

Effect of Coadministered Glucose - 6 - Phosphate on the Susceptibility to Phosphonomycin, *In Vitro* and *In Vivo*, of a Wild-Type, Inducible Bacterial Strain and A Non-Inducible Mutant Derived Therefrom.

A mutant designated *2017 A* showing no additional response *in vitro* to phosphonomycin upon addition of glucose - 6 - phosphate was isolated from its parent, the naturally occurring pathogen *Escherichia coli 2017* by the procedure described in Example 6 for the isolation of Strain *MB 2489 A2* from its parent MB 2489. Mutant *2017 A* showed a normal ability to metabolise glucose - 6 - phosphate. Its *in vitro* susceptibility to phosphonomycin, *in vivo*, relative to the parent strain, was determined twice in mouse protection trials described below, by the protocol established in Example 9.

IN VITRO SUSCEPTIBILITY TESTS

	Zones of inhibition (mm) surrounding discs bearing 30 μg of phosphonomycin alone, or in combination with 5 μg of glucose-6-phosphate, dosodium.	
	Phosphonomycin alone	plus glucose -6-phosphate
E. coli 2017	18	27
E. coli 2017 A	17.5	18

CURATIVE EFFICACY OF PHOSPHONOMYCIN IN INFECTED MICE

(ED_{50}'s in mg)

	Phosphonomycin alone	Phosphonomycin co-administered with 1 mg glucose-6-phosphate, disodium
TRIAL I		
E. coli 2017		
1×10^6 cells	0.230	0.015
= 9 LD_{50}'s		
E. coli 2017 A		
1×10^6 cells	0.166	0.284
= 7 LD_{50}'s		
TRIAL II		
E. coli 2017		
5×10^6 cells	0.821	0.021
= 13 LD_{50}'s		
E. coli 2017 A		
9×10^6 cells	1.420	1.130
= 33 LD_{50}'s		

The indifference of mutant *2017 A* to the combination of glucose - 6 - phosphate with phosphonomycin *in vitro*, is reflected perfectly *in vivo* by the failure of co-administered glucose - 6 - phosphate to enhance the efficacy of phosphonomycin in treatment of mice infected by this mutant. Thus the normal potentiation by glucose - 6 - phosphate of phosphonomycin's curative effect must be attributed to its direct action on inducible infecting strains of bacteria and not by any host response that might be conjectured (such as enhanced drug absorption or immune response) which would have affected the mutant as favorably as the parent strain. The similar efficacy of phosphonomycin alone, against infections due to inducible and non-inducible organisms, implies that endogeneous inducers are either absent from or at too low a level (in those areas of the body invaded by microorganisms) to evoke the biosynthesis of the hexose - 6 - phosphate transport system. Thus the manifest potential benefits resulting from induction of this system, require explicit administration of exogeneous inducer by the therapist.

WHAT WE CLAIM IS:—

1. A method of potentiating the antibacterial activity of a phosphonomycin antibiotic in a non-human animal, that comprises bringing bacteria into contact with an inducer selected from phosphatides, sugar phosphates and salts thereof, and polyhydric alcohols, whereby an existing phosphonomycin transport pathway in the bacteria is enhanced or a new pathway is brought into being, and subsequently or simultaneously bringing the bacteria into contact with a phosphonomycin antibiotic.

2. A method as claimed in claim 1 in which the inducer is glucose - 6 - phosphate.

3. A method as claimed in claim 1 in which the inducer is mannose - 6 - phosphate.

4. A method as claimed in claim 1 in which the inducer is glucose - 1 - phosphate.

5. A method as claimed in claim 1 in which the inducer is ribose - 5 - phosphate.

6. A method as claimed in claim 1 in which the inducer is galactose - 6 - phosphate.

7. A method as claimed in claim 1 in which the inducer is lactose.

8. A method as claimed in claim 1 in which the inducer is glycerol, *DL* - α - glycerophosphate, glucose - 6 - phosphate disodium or dipotassium, fructose - 6 - phosphate disodium, mannose - 6 - phosphate disodium, 2 - deoxyglucose - 6 - phosphate disodium, 2 - amino-2 - deoxy - glucose - 6 - phosphate disodium, ribose - 5 - phosphate disodium, phosphatidyl ethanolamine, glucose - 1',6' - diphosphate tetrapotassium pentahydrate, glucose - 1 -phosphate disodium, 5 - phosphoryl - ribose - 1-pyrophosphate dimagnesium dihydrate, riboflavin - 5 - phosphate disodium, galactose - 6-phosphate disodium or a glucose - 6 - phosphate *n* - octylammonium salt.

9. A method as claimed in any one of claims 1—8 in which the phosphonomycin antibiotic is a salt of phosphonomycin.

10. A method as claimed in any one of claims 1—9 in which the inducer and the antibiotic are administered parenterally in a single composition.

11. A method as claimed in any one of claims 1—9 in which the inducer is administered parenterally and the phosphonomycin antibiotic orally.

12. An antibiotic composition comprising a phosphonomycin antibiotic as herein defined an inducer of a phosphonomycin transport system in bacteria, selected from phosphatides, sugar phosphates and salts thereof, and polyhydric alcohols, and a pharmaceutically acceptable carrier, diluent or vehicle.

13. A composition as claimed in claim 12, in the form of tablets, powders, granules, capsules, suspensions, solutions, elixirs, syrups or sterilized solutions or suspensions for parenteral administration.

14. A composition as claimed in claim 12, in topically administrable form.

15. A method as claimed in claim 1, substantially as hereinbefore described in any one of Examples 1—17.

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